

SUPPLEMENTAL TABLE S1**Proteins co-purify with MIX tagged complex and are considered as non-specific contaminants of TAP tag purifications**

Protein_Name	Description
Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative
Tb11.02.4150	PPDK pyruvate phosphate dikinase
Tb11.02.5500	glucose-regulated protein 78, putative
Tb927.6.4280	GAPDH glyceraldehyde 3-phosphate dehydrogenase, glycosomal
Tb927.7.210	proline oxidase, putative
Tb10.389.0690	mitochondrial carrier protein, putative
Tb927.3.1840	3-oxo-5-alpha-steroid 4-dehydrogenase, putative
Tb09.160.4310	GDH 28G16.445 glutamate dehydrogenase
Tb927.7.7420	ATP synthase alpha chain, mitochondrial precursor
Tb10.70.5670	TEF1 elongation factor 1-alpha
Tb10.61.1820	mitochondrial carrier protein, putative
Tb11.01.3110	heat shock protein 70
Tb11.02.0750	TCP-1-zeta t-complex protein 1, zeta subunit, putative
Tb10.61.2070	RPS2 40S ribosomal protein S2, putative
Tb927.2.3030	10C8.315 ATP-dependent Clp protease subunit, heat shock protein 78 (HSP78), putative
Tb10.70.2650	elongation factor 2
Tb927.3.1380	TAP102 ATP synthase beta chain, mitochondrial precursor
Tb10.70.3290	DHH1 ATP-dependent DEAD-box RNA helicase, putative
Tb927.8.7530	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor, putative
Tb09.160.3270	1L12.525 eukaryotic initiation factor 4a, putative
Tb927.5.3800	glutamine hydrolysing (not ammonia-dependent) carbomoyl phosphate synthase, putative

SUPPLEMENTAL TABLE S2**Proteins co-purify with cyt c oxidase tagged complexes and are considered as non-specific contaminants of TAP tag purifications**

Protein_Name	GeneDB_Annotation
Tb927.6.4280	GAPDH glyceraldehyde 3-phosphate dehydrogenase, glycosomal
Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative
Tb927.5.1060	TAP067 mitochondrial processing peptidase, beta subunit, putative
Tb927.3.1840	3-oxo-5-alpha-steroid 4-dehydrogenase, putative
Tb927.3.1380	TAP102 ATP synthase beta chain, mitochondrial precursor
Tb11.02.4150	PPDK pyruvate phosphate dikinase
Tb11.01.3110	heat shock protein 70
Tb10.70.5670	TEF1 elongation factor 1-alpha
Tb10.70.5110	mMDH mitochondrial malate dehydrogenase
Tb10.61.1820	mitochondrial carrier protein, putative
Tb10.389.0690	mitochondrial carrier protein, putative
Tb09.211.3560	glk1 gk glycerol kinase, glycosomal
Tb09.160.4310	GDH 28G16.445 glutamate dehydrogenase

SUPPLEMENTAL TABLE S3

Proteins identified variably in TAP-tagged complexes indicating that they associate transiently with cyt c complex or they may not be part of the complex

<i>T.brucei</i> gene	TM	MW ^b	TAP MIX	TAP COXIV	TAP COXV	TAP Tb2320	TAP Tb1900	Possible function/similarity
Tb927.3.750	1	37.8	1	√	√	√	1	Hypothetical protein; conserved; SET domain
Tb927.3.1410*	NI	19.3	ND	√	√	√	1	Cyt c oxidase subunit VII; Tryp specific
Tb927.7.5260	NI	22.7	ND	1	√	√	ND	Hypothetical protein; Tryp specific
Tb927.8.6080	NI	38.1	ND	1	√	√	√	Hypothetical protein; conserved
Tb09.211.4400	NI	48.9	ND	√	√	1	ND	Hypothetical protein; Tryp specific
Tb10.70.0625*	1	12.3	1	√	√	√	ND	Hypothetical protein; Tryp specific
Tb10.70.7840	1	56.9	1	√	√	√	ND	Cholin dehydrogenase
Tb11.01.5390	1	36.1	ND	1	√	√	√	DnaJ chaperon
Tb11.02.1440	2	36.3	ND	ND	1	√	1	Hypothetical protein; conserved
Tb11.02.2280	NI	40	1	ND	√	1	1	Surfeit 1
Tb11.02.2480	NI	25.8	ND	1	1	√	1	Hypothetical protein; Tryp specific
Tb927.2.3180	NI	114	1	ND	ND	ND	ND	Pet 309; cox translation activator
Tb927.4.4620*	1	18.7	ND	1	1	1	ND	Cyt c oxidase subunit VIII; Tryp specific
Tb10.100.0160*	NI	19.2	ND	ND	ND	1	ND	Cyt c oxidase subunit VI
Tb10.6k15.2180*	1	13.8	ND	ND	ND	1	ND	Cyt c oxidase subunit IX; Tryp specific
Tb10.70.4380	1	27	ND	1	1	1	ND	Cox 11; cox assembly factor
Tb11.01.3780	8	43.5	1	ND	1	1	ND	Cox 15; cox assembly factor

TM – indicates number of predicted transmembrane domains calculated by TMHMM 2.0 software; NI – no transmembrane domain identified

^b In kDa.

√ – indicates that the number of unique peptides detected with each of the tandem affinity purification-tagged constructs is higher than 2.

ND – protein has not been detected

* – proteins identified in *L. tarentolae* and *C.fasciculata* cyt c oxidase complexes (Horváth et al., 2000, Speijer et al., 1996)

SUPPLEMENTAL TABLE S4

Genomic analysis of mitochondrial components of cytochrome c oxidase from *T. brucei*. Identification of homologous sequences in eukaryotic organisms.

<i>Homo sapiens</i>	<i>Saccharomyces cerevisiae</i>	<i>Arabidopsis thaliana</i>	<i>Trypanosoma brucei</i>	Homology	GeneDB annotation	Presence in tagged cytochrome c oxidase complexes
1. core subunits						
COI	COI	AtMg01360	COI			
COII	COII	AtMg00160	COII			
COIII	COII	AtMg00730	COIII			
COXIV	COX5	-	Tb927.4.4620	+	COVIII	√
COXVa	COX6	-	-			
COXVb	COX4	AT3g15640, At1g80230 At3g62400; At2g47380	-			
-	-	At4g37830	-			
COXVIa	COX13	AT1g22450	-			
COXVIb	COX12	-	Tb10.100.0160	+	COVI	√
COXVIc	COX9	-	-			
COXVIIa	COX7	-	-			
COXVIIb	-	-	-			
COXVIIc	COX8	-	-			
COXVIII	-	-	-			
-	-	-	Tb927.4.4100		trCOIV	√√
-	-	-	Tb09.160.1820		COV	√√
-	-	-	Tb927.3.1410		COVII	√
-	-	-	Tb10.6k15.2180		COIX	√
-	-	-	Tb11.01.4702		COX 10	√√
-	-	-	Tb10.70.1890		HP	√√
-	-	-	Tb09.211.4740		HP	√√
-	-	-	Tb09.211.1900		HP	√√
-	-	-	Tb927.7.6990		HP	√√
-	-	-	Tb11.01.1900		HP	√√
-	-	-	Tb11.46.0006		HP	√√
2. assembly factors						
Surfeit1	SHY I	At3g17910	Tb11.02.2280	++	HP	√

SCO1/SCO2	SCO1/SCO2	AT3g08950	Tb09.160.1140	++	electron transport protein SCO1/SCO2	√√
			Tb09.211.0510	++	cytochrome c oxidase assembly factor	-
			Tb927.1.1580	++	electron transport protein SCO1/2,	-
COX10	COX10	At2g44520	Tb927.5.1310	++	protoheme IX farnesyltransferase	-
COX11	COX11	AT1g02410	Tb10.70.4380	++	cytochrome C oxidase assembly protein	√
COX15	COX15	AT5g56090	Tb11.01.3780	++	cytochrome oxidase assembly protein	√
AAH01702	COX16	AT4g14145	-			
COX17	COX17	At1g53030	Tb927.3.2650	+	cytochrome c oxidase copper chaperone	-
OXA	COX18,OXA1	At5g62050	Tb11.02.4020	++	HP	-
			Tb09.211.1850	++	HP	-
COX19	COX19	AT1G66590	Tb10.61.0510	++	HP	-
NP_077276, Q9BUK0 AAH47722, Q86WW8	COX23, COX17	At1g02160	-			
	PET191	-	-			
		PPRC protein family				
LRPPRC	PET309		Tb927.2.3180	++		√
-	PET111	-	-			
-	PET122	-	-			
-	PET117	-	-			
-	PET100	AT4g14615	-			
-	-	AT4g37830	-			
-	COX20	-	-			
-	COX14	-	-			

- homologous sequence has not been identified

√√- identified in all four TAP tags experiments,

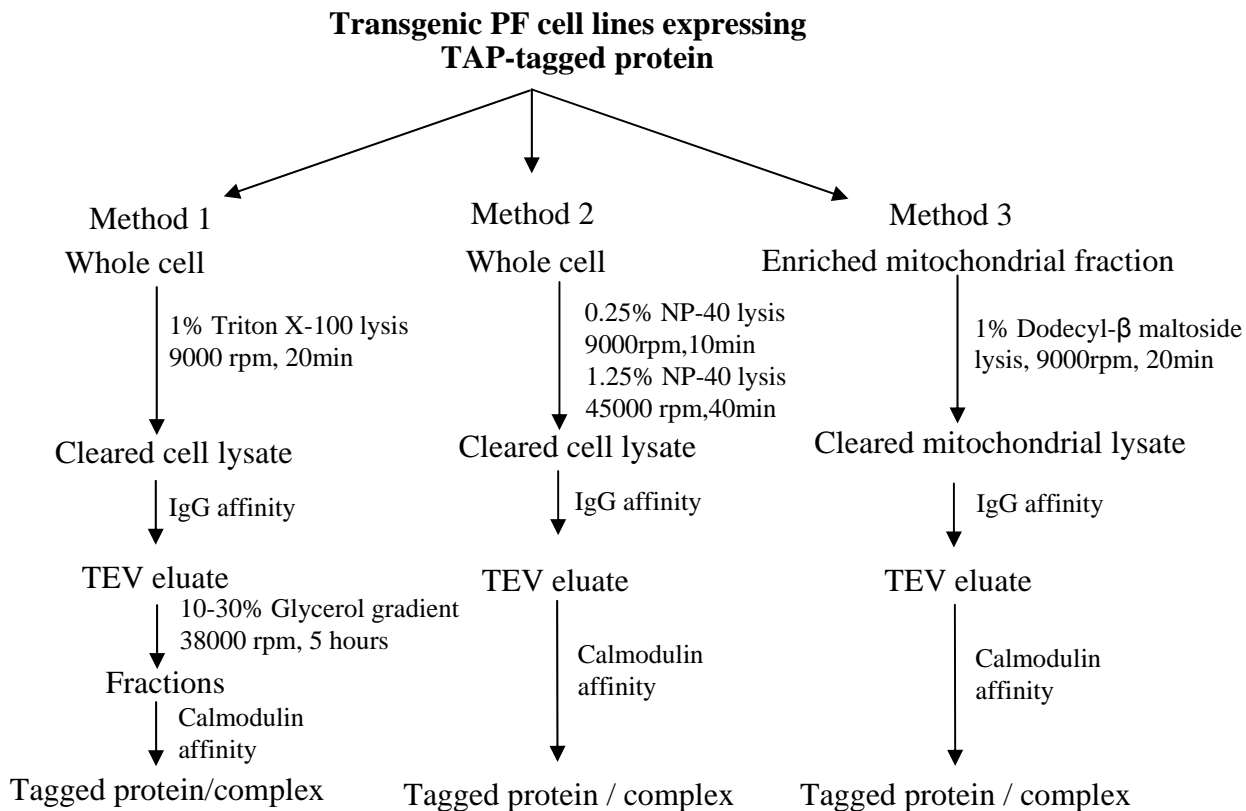
√- identified in less than four TAP tags experiments,

+ - *E*-value higher than 1e-03

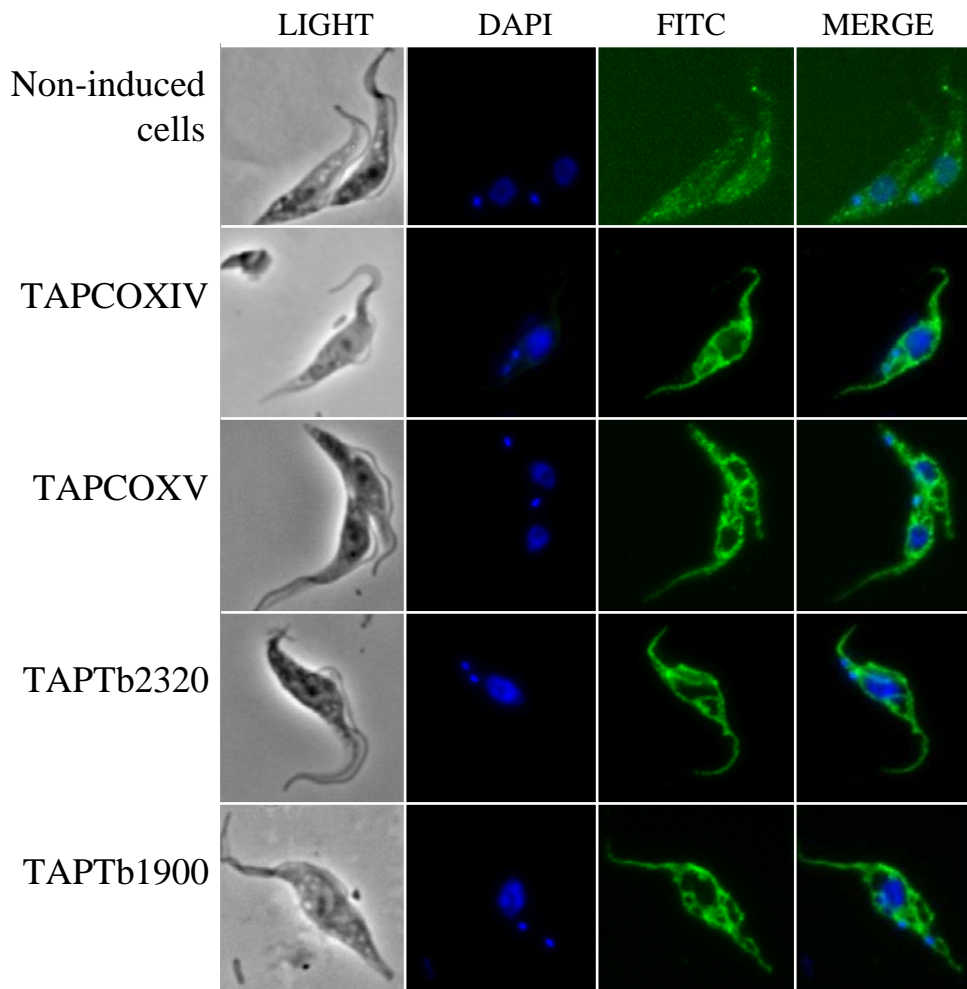
++ - *E*-value lower than 1e-03

HP - hypothetical protein

Supplemental figure S1
Schematic presentation of the methods used for purification of tagged complexes.



Supplemental figure S2
Immunofluorescence assay of expressed TAP-tagged proteins.



Immunofluorescence assay. Subcellular localization of the expressed tagged proteins within the cell was determined by immunofluorescence assay (IFA) using anti-*myc* mAb (Invitrogen). Briefly, the cells were fixed with 4% formaldehyde, permeabilized with 0.2% Triton X-100, blocked with 5% FBS, and incubated with anti-*myc* antibody at 1:100 dilution. After washing the cells were incubated with anti-mouse FITC conjugated antibody (1:250 dilution) (SIGMA), washed and treated with DAPI stain (4,6-diamidino-2-phenylindole) to visualize DNA. Phase contrast image of the cells and their fluorescence was captured with a Nikon fluorescence microscope equipped with camera and the appropriate filters.